



PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12N 15/10		A1	(11) International Publication Number: WO 00/65041																													
			(43) International Publication Date: 2 November 2000 (02.11.00)																													
(21) International Application Number: PCT/CA00/00424 (22) International Filing Date: 20 April 2000 (20.04.00) (30) Priority Data: 2,270,106 23 April 1999 (23.04.99) CA (71)(72) Applicant and Inventor: HAJ-AHMAD, Yousef [CA/CA]; 344 Merritt Street, St. Catharines, Ontario L2T 1K6 (CA). (74) Agents: LEDGLEY, Cynthia, J. et al.; Dimock Stratton Clarizio, 20 Queen St. West, Suite 3202, Box 102, Toronto, Ontario M5H 3R3 (CA).		(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>																														
(54) Title: PROCESS FOR NUCLEIC ACID PURIFICATION USING SILICON CARBIDE																																
<table border="1"> <thead> <tr> <th></th> <th colspan="2">Roche</th> <th colspan="2">Promega</th> <th colspan="2">Clontech</th> <th colspan="2">Norgen</th> </tr> <tr> <th></th> <th>M</th> <th>A</th> <th>B</th> <th>A</th> <th>B</th> <th>A</th> <th>B</th> <th>A</th> <th>B</th> </tr> </thead> <tbody> <tr> <td></td> <td colspan="9"> </td> </tr> </tbody> </table> <p>Comparison of digestions of plasmid pCMV beta (7164 bp) purified using three commercial DNA purification kits and silicon carbide 1000. "A" lanes show undigested pCMV beta while "B" lanes show pCMV beta digested with <i>Hind</i> III. Lane "M" is a marker lane. Commercial kits used for purification are listed along top of figure.</p>					Roche		Promega		Clontech		Norgen			M	A	B	A	B	A	B	A	B										
	Roche		Promega		Clontech		Norgen																									
	M	A	B	A	B	A	B	A	B																							
(57) Abstract																																
<p>The invention disclosed is particularly directed to a process for the purification of DNA, and is especially useful for the isolation of biologically active plasmid DNA. The process is initiated by binding the DNA to silicon carbide particles, either in the presence or absence of chaotropic agents. The bound DNA is subsequently eluted from the resin in low salt buffer or water. The resulting purified DNA is substantially free of proteins and is shown to be biologically active. The scope and diversity of the process is demonstrated here since this process can be used for the isolation of RNA and genomic DNA as well as the purification of PCR products and isolation of DNA from agarose gel slices.</p>																																

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

PROCESS FOR NUCLEIC ACID PURIFICATION USING SILICON CARBIDE

FIELD OF THE INVENTION

The invention relates to methods for nucleic acid purification and in particular to purification methods wherein nucleic acid
5 is removed from a solution by binding it to an inert carrier.

BACKGROUND OF THE INVENTION

It is well known that the purity of DNA and RNA used in scientific and medical research is a critical factor in the success of such research. For example, gene transfer
10 experiments in cell cultures, immunization using DNA-based vaccines and DNA sequencing projects are a few of the research areas where DNA of high purity is essential for project success. Sufficiently pure DNA is not normally attainable using traditional methods of DNA isolation. Furthermore, the
15 process of isolating and purifying DNA is a major rate-limiting step in molecular biology. Traditional methods of isolating pure DNA have typically involved toxic chemicals such as phenol/chloroform extraction procedures, and very long centrifugation times such as in Cesium Chloride banding
20 procedures. Not only are such procedures slow and costly, they also represent a health risk to laboratory staff and demand the usually expensive disposal of hazardous chemicals. Furthermore, despite careful preparation, DNA prepared using these methods may not always be free of RNA, proteins and
25 chromosomal DNA.

As a result, the demand for safe, high quality and rapidly obtainable DNA preparations grown in bacteria such as *E. coli* has risen steadily and a variety of commercial kits have become available to meet a wide range of DNA purification
30 needs. However, a number of these kits suffer from disadvantages, ranging from high price to failing to produce the desired quality and yield.

It is known that DNA will bind to silicon-containing materials such as glass slurries and diatomaceous earth. In fact, DNA purification kits are available which make use of these silicon-containing substances. For example, Bio 101 offers the GENECLLEAN™ kit which makes use of a glass slurry and sodium iodide (binding buffer) and BioRad™ offers a plasmid purification kit using diatomaceous earth (Celite™) suspended in guanidine hydrochloride buffer.

U.S. Patent Nos 5,503,816 and 5,525,319 and 5,693,785 by Woodard et al. describe the use of silicate compounds for DNA purification. The disadvantage with these materials is that the required silicate material is not readily commercially available in the appropriate form, and typically must be prepared requiring additional time for DNA isolation procedures.

U.S. Patent Nos 5,438,129 and 5,625,054 describe DNA isolation procedures which utilize flourinated Celite™, flourinated silicon dioxide or flourinated aluminum hydroxide. These inventions require the use of toxic chemicals to create the flourinated surfaces to which the DNA will bind. In addition, chaotropes which are also toxic, are still required for these procedures.

U.S. Patent Nos 5,534,054 and 5,705,628 both disclose methods for isolating DNA which do not require the use of toxic chaotropic agents. The former patent discloses the use of silicon tetrahydrazide for the purification of DNA, but the preparation of the binding material requires the use of toxic chemicals which can lead to conditions such as nausea and temporary blindness. The latter of the patents relates to a non-specific, reversible binding of DNA particles using magnetic microparticles.

SUMMARY OF THE INVENTION

It is now an object of the present invention to provide a method for the purification of DNA using an inexpensive commercially available material, that does not require the use of chaotropic agents, and rapidly yields high quality preparations of DNA.

Accordingly, the invention provides the use of silicon carbide for binding a nucleotide polymer.

The invention further provides a method for purifying nucleic acid from a sample including the steps of

- providing a silicon carbide carrier for binding nucleic acids;
- adding the nucleic acid containing sample to the carrier for binding nucleic acid in the sample to the silicon carbide;
- separating the silicon carbide from the liquid;
- eluting from the silicon carbide the nucleic acid bound thereto.

The present invention provides an economical nucleic acid purification method using silicon carbide compounds, preferably commercially available industrial quality silicon carbide. A typical industrial preparation of silicon carbide (SiC) which is applicable for use in the purification is composed of 97.8% silicon carbide and small amounts of silicon dioxide, silicon, iron, aluminum and carbon. This substance is affordable and readily available as a DNA-binding material. Silicon carbide is available in a variety of grit sizes or grades, and each grade has a different capacity for binding nucleic acids (all obtained through Ritchey Supply Ltd. Mississauga, Ontario). Any grade of SiC used in the method according to the present invention is preferably suspended as a 15% (w/v) slurry, preferably in either distilled water or a solution of guanidine hydrochloride.

Another preferred process of the invention is used for the purification of plasmid DNA from a sample and involves the following steps:

1. Immobilizing the DNA in the sample onto silicon carbide 1000 in the presence or absence of a binding buffer;
2. Separating the silicon carbide with the DNA immobilized thereon from the sample;
3. Washing the subsequent silicon carbide-bound DNA with an ethanol-containing buffer;
4. Removing the ethanol-containing buffer; and
5. Eluting the DNA in a low salt buffer (TE) or in water.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an agarose gel illustrating the effect of pre-washing silicon carbide.

Figure 2 shows a result of using the invention for the isolation of DNA fragments from agarose gel slices.

Figure 3 is a picture of agarose gel demonstrating the recovery of PCR products using the invention.

Figure 4 is an agarose gel photograph showing the improved isolation of *E. coli* DH5 α genomic DNA using silicon carbide.

Figure 5 shows an agarose gel indicating isolation of RNA using the process of the invention.

Figure 6 is an agarose gel showing a range of plasmids, or different sizes isolated using the invention.

Figure 7 is an agarose gel comparing the quality of the DNA isolated using an embodiment of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

All grades of silicon carbide tested were able to purify plasmid DNA. Furthermore, all molarities of guanidine hydrochloride (including 0M or simply distilled water) appeared to be sufficient to allow the binding of DNA to silicon carbide. However, two trends were evident:

1. Finer grade silicon carbide (i.e. smaller grit size, such as SiC 1000) appeared to give higher yields of bound plasmid DNA; and

- 5 -

2. A high concentration of guanidine hydrochloride (5-7M) produced higher plasmid yield. Thus most of the examples illustrated in this report involve the use of SiC 1000 suspended in a 5-7 molar guanidine hydrochloride solution.

5 The primary objective of the exemplary methods carried out was the purification of plasmid DNA from bacterial lysates by immobilizing DNA onto silicon carbide 1000 in the presence of a high salt binding buffer such as guanidine hydrochloride. The bound DNA was subsequently eluted into a low salt buffer
10 such as TE buffer or water.

Generally, the first step in the isolation of plasmid DNA, using the preferred method of this invention, is the pelleting of 1-3 ml of an overnight bacterial culture comprised of host cells containing a plasmid of interest. This is followed by
15 the resuspension of the bacterial pellet in a buffer of Tris HCl and EDTA including 100µg/ml RNase A. The presence of RNase A in this buffer significantly reduces the amount of RNA generally observed in the final plasmid preparation. The cells are then lysed under alkaline conditions (NaOH and
20 sodium dodecyl sulphate (SDS)) until the tubes containing resuspended cells are largely clear (generally not longer than five minutes is required). The solutions are then neutralized using 2M potassium acetate and centrifuged for at least 10 minutes. After this stage, the solution containing plasmid
25 DNA is brought into contact with the silicon carbide and binding of plasmid to SiC occurs while proteins and other macromolecules generally do not bind. Subsequently, during the wash steps that follow, plasmid DNA remains bound to SiC while proteins are removed as wash is aspirated or flows
30 through a spin column filter. After generally two rounds of washing, the resin bed (in a spin filter column) or resin pellet (in a centrifuge tube) is washed with low salt buffer (TE) or water generally warmed to 65°C to elute the plasmid DNA. Larger volumes of elution buffer will tend to release
35 more DNA from silicon carbide, however, this also tends to

result in much lower concentrations of final DNA preparation. Also, two rounds of smaller elution volumes tends to elute more DNA than one large elution volume. For example, eluting two times with 50 μ l each time rather than once with 100 μ l
5 tends to yield larger quantities of plasmid DNA.

The process of plasmid DNA isolation and the isolation of other nucleic acids is further illustrated with the following examples. These examples are designed to illustrate the scope and diversity of the invention but are not intended to limit
10 the range of the invention. Indeed, not all possible examples are presented here.

Silicon Carbide is a dark grey, crystalline substance which is insoluble in water, acids and alkalines. Commercial preparations of silicon carbide can be obtained, wherein they
15 are typically composed of greater than 98% SiC with smaller amounts of carbon (C), silicon (Si), silicon dioxide (SiO₂), and iron (Fe) also present. Silicon carbide is also available in a variety of grit sizes or grades. One preferred embodiment of the invention utilizes a grade of silicon carbide known as
20 the 1000 grade with an average particle size of 4.5 microns. The silicon carbide used was manufactured by EXOLON ESK (Tonawanda, NY, USA) and distributed through Ritchey Supply Ltd (Mississauga, Ontario, Canada).

Example 1

25 Preparation of Silicon Carbide 1000 Slurry

In a preferred embodiment of the invention a 15% weight/volume (w/v) slurry of silicon carbide 1000 grade grit is prepared in either distilled water or a binding buffer of guanidine hydrochloride. In this preparation silicon carbide is
30 sterilized using an autoclave and subsequently measured without any wash or refinement of the commercial silicon carbide preparation. This prepared silicon carbide resin is then added to the liquid in which it is to be suspended, typically either distilled water or a 7M guanidine

hydrochloride solution. For larger scale trials a 15% (w/v) suspension of silicon carbide in solution was typically prepared by adding 15 grams of the sterilized silicon carbide to 100 ml of solution whereas for smaller scale trials the 15% silicon carbide suspension would be prepared by adding 1.5 grams of the silicon carbide to 10 ml of solution. Slurries produced in this manner were mixed well prior to any use in purification protocols and stored at room temperature when not in use.

10

Example 2**Plasmid DNA Purification Protocol**

There are different protocols which can be followed for the purification of plasmid DNA using a purification process in accordance with the invention and as binding agent a slurry as described herein. These protocols could involve the use of a pelleting step, or a spin column. The composition of the buffers used in the investigation of preferred embodiments of this invention were as set out below:

1. Cell Resuspension Solution:
 - 20 50 mM Tris-HCl, pH 7.5
 - 10 mM EDTA
 - 100 µg/ml RNase A (ribonuclease A, DNase-free)
2. Cell Lysis Solution:
 - 0.2 M NaOH
 - 25 1% SDS (sodium dodecyl sulfate)
3. Neutralization Solution:
 - 2 M Potassium Acetate, pH 4.8
4. Wash Solution
 - 20 mM Tris HCl, pH 7.5
 - 30 5 mM EDTA
 - 50 % EtOH (ethanol)
5. TE Buffer
 - 10 mM Tris HCl
 - 1 mM EDTA

In order to purify *E. coli* plasmid DNA the initial step is the production of a cleared lysate from, typically 1.0 - 3.0ml, of a culture of *E. coli*. The bacterial culture is pelleted by centrifugation at 14,000rpm for 1 minute, and the supernatant is then removed. Approximately 250µl of cell resuspension solution is added to the pellet, which is then resuspended by vortexing vigorously. Once the cells have been resuspended, 250µl of cell lysis solution is added to the suspension, which is then mixed by several gentle inversions, but without resort to a vortex. The resulting mixture can then be incubated for up to 5 minutes at 37°C until the solution becomes clear. Once the solution has cleared, no longer than 5 minutes, 350µl of Neutralization solution are added and again mixed by gentle inversion several times. The resulting mixture is then centrifuged at 14,000 rpm for 10 minutes to pellet a white precipitate. The supernatant representing the cell lysate is carefully transferred to a fresh Eppendorf tube. 200µl of the silicon carbide 1000 grade slurry, prepared as above, is added to the tube and mixed therewith by inversion or vortex.

If the plasmid DNA is to be isolated using a pelleting technique the tube is then centrifuged for 1 minute at 14,000rpm. The resulting supernatant is then aspirated, while taking care not to disturb the silicon carbide pellet. 500µl of the Wash Solution is added to the pellet and vortexed well. The resulting suspension is again centrifuged for 1 minute at 14,000rpm. The supernatant is thoroughly aspirated. This washing and centrifugation step is then repeated using a further 500µl of Wash Solution. The supernatant is again thoroughly aspirated. If the pellet appears to be moist, it can be dried by placing the Eppendorf in a 37°C oven or even on the benchtop for a few minutes.

100µl of TE buffer or distilled water warmed to 65°-70°C are then added to the dried pellet in the Eppendorf and incubated at 65°-70°C for 1 minute. The Eppendorf tube is then

centrifuged at 14,000rpm for 1 minute. The supernatant, which contains the eluted DNA is carefully pipetted from the resin pellet into a fresh Eppendorf tube. It is important to attempt to transfer as little of the resin into the final preparation as is possible.

If the plasmid DNA is to be isolated using a Spin Column technique the cell lysate and the silicon carbide slurry are mixed by pipetting, then transferred to a spin column filter which has been inserted into a fresh Eppendorf tube. The pore size of the spin column filter should typically be in the range of between 0.22 microns to 0.45 microns, though it can be slightly larger since the average particle size of the silicon carbide 1000 grade is 4.5 microns. The Eppendorf with the spin column is then centrifuged at 14,000rpm for 30 to 60 seconds. The flow through is discarded and 500µl of Wash Solution is added to the spin column. The spin column is again centrifuged at 14,000rpm for 30 to 60 seconds. Again the flow through is discarded and a further 500µl of Wash Solution added. The spin column is centrifuged at 14,000rpm for 1-2 minutes to ensure the removal of all traces of wash from the spin column. The flow-through is discarded once more and the spin column is then placed in a fresh Eppendorf tube. 100µl of TE buffer or water warmed to 65°-70°C is added to the spin column which is subsequently incubated for 1 minute. The spin column is centrifuged at 14,000rpm for 1 minute. The TE buffer elutes the DNA so that the centrifugation results in eluted DNA being washed through into the Eppendorf tube ready for use.

Example 3

Effect of Washing Silicon Carbide 1000 Prior to Use as a DNA
Binding Substance

The effect of washing the silicon carbide resin, prior to making the 15% (w/v) slurry, on the digestability of DNA purified in a pellet format was also examined to determine a preferred embodiment. Silicon Carbide 1000 grade was either not washed, washed once, twice or three times with 1M guanidine hydrochloride at room temperature, or distilled water at room temperature or distilled water at 80°C as outlined in the following protocol:

1. Place 1.5 grams of silicon carbide 1000 grade resin in a 15ml tube.
2. Suspend the silicon carbide resin in 10ml of the desired solution (either distilled water or 1M guanidine hydrochloride) and vortex.
3. Place on an aliquot mixer for five minutes.
4. Centrifuge for 5 minutes at 3700 rpm to compact pellet of resin.
5. Discard the supernatant liquid and repeat from step 2 for repeated washes.
6. After the desired number of washes, resuspend the silicon carbide resin in 10 ml of autoclaved distilled water.
7. Isolate plasmid DNA using the protocol outlined above.

Twelve samples of pUC19 DNA were purified using the protocol outlined in Example 2 where the Silicon Carbide resin used for binding DNA varied according to the number of wash cycles it was treated with prior to use. DNA purified using resin prepared from twelve different washes demonstrated digestion with the restriction enzyme *Hind* III (Figure 1).

Example 4**Scale-up of Purification Protocol**

The isolation method disclosed in this invention can be scaled up to accommodate larger quantities of plasmid DNA. The
5 plasmids pUC19 (2686 bp) and pCMV beta (7164 bp) were used in the example protocol described below. The silicon carbide slurry used was 7.5 grams of silicon carbide 1000 grade in 50 ml distilled water or 7M Guanidine Hydrochloride. The protocol followed the identical steps to those outlined in the small
10 scale protocol previously described, though using the amounts of components as listed in Table A.

TABLE A. Examples of Scale-up

Culture Volume	Resuspension Buffer	Lysis Buffer	Neutralization Buffer	Silicon Carbide Slurry	TE Buffer	Tube needed
1.5-3.0 ml	250 µl	250 µl	350 µl	200 µl	100 µl	1.5 ml
50 ml	4 ml	4 ml	4 ml	3 ml	500 µl	50 ml
100-500 ml	15 ml	25ml	15 ml	10 ml	2-4 ml	250 ml

15 DNA purified using scale-up protocols, such as are illustrated in TABLE A, was found to be of comparable quality to small-scale preparations described earlier. However, no simple ratio of components was found to be optimal for scale-up purposes, such that optimization for larger scale preparations will
20 depend upon the culture volume being used.

Example 5

Isolation of DNA from Agarose Gel Slices

- This example illustrates the isolation of a fragment of DNA from an agarose gel by binding onto Silicon Carbide 1000. The silicon carbide slurry used in this example was 7.5 grams of silicon carbide 1000 grade in 50 ml of 7M guanidine hydrochloride. Two samples of the plasmid pCMVbeta (7164 bp) were digested with the restriction enzyme *Hind* III and loaded onto an agarose gel (1% (w/v) agarose gel in TAE buffer). After electrophoresis, the gel was stained with SYBR Gold and photographed (Figure 2, left side photo). The 7164 bp bands of pCMV beta were excised from the gel as precisely as possible using a razor blade and DNA was isolated from the agarose slices using the following modification of Example 2:
1. The excised bands were placed in fresh Eppendorf tubes.
 2. 400 μ l of 7M Guanidine Hydrochloride solution (without resin) was added to the gel slice.
 3. The agarose slice was heated at 65°C until completely melted.
 4. 50 μ l of silicon carbide 1000 slurry (prepared as in Example 1, described above) was added to the melted agarose.
 5. The tube was vortexed well to mix and incubated for 5 minutes at room temperature.
 6. The mix was then centrifuged for 1 minute at 14,000 rpm.
 7. The supernatant was carefully aspirated.
 8. 500 μ l Wash Solution was added and the tube was vortexed well.
 9. The tube was centrifuged at 14,000 rpm for 1 minute.
 10. The wash was carefully aspirated.
 11. The wash step was repeated and aspirated as before.
 12. The resin was allowed to dry for a few minutes.
 13. The DNA fragment was eluted from the resin by adding 50 μ l of TE buffer warmed to 65°C.
 14. The tube was centrifuged at 14,000 rpm for 1 minute.

- 13 -

15. The eluted DNA was pipeted off the resin and run on an agarose gel (Figure 2., right side photo).

Example 6

Purification of DNA Amplified by PCR

- 5 This example illustrates the use of a silicon carbide 1000 slurry (as prepared in Example 1) in the purification of DNA amplified by PCR. The purification protocol begins with a completed PCR reaction in a PCR tube. If mineral oil was used to cap the reaction volume, the aqueous (lower) phase must be
- 10 carefully removed to a fresh Eppendorf tube. It is to this aqueous phase of the reaction volume that the method of the invention was applied using the following protocol:
1. Add 7M Guanidine Hydrochloride solution to bring volume present in tube to approximately 100 μ l.
 - 15 2. Add 50 μ l silicon carbide 1000 slurry (as prepared in Example 1) and vortex to mix.
 3. Incubate 5 minutes at room temperature.
 4. Centrifuge for 1 minute at 14,000 rpm.
 5. Carefully aspirate the supernatant.
 - 20 6. Wash with 500 μ l Wash Solution and vortex to mix.
 7. Centrifuge for 1 minute at 14,000 rpm.
 8. Carefully aspirate the wash and repeat the wash step.
 9. Dry pellet for a few minutes.
 10. Elute PCR products with TE buffer warmed to 65°C.
 - 25 11. Spin at 14,000 rpm for 1 minute and carefully pipet the eluted PCR products into a fresh Eppendorf tube.

PCRs were carried out and a sample of the products was resolved by agarose gel electrophoresis (Figure 3, left side photo). To the remaining reaction volume, the above protocol

30 was applied and the PCR products isolated were subsequently run on an agarose gel to observe recovery (Figure 3, right side photo). The results indicate that the PCR products observed on the initial test gel were observed to be isolated using silicon carbide 1000 method. These results demonstrate

the protocol's usefulness in separating PCR products from amplification primers, primer-dimers, and enzymes.

Example 7

Isolation of Genomic DNA from *E. coli* DH5 α

- 5 This example illustrates the use of the present invention in the binding and isolation of genomic DNA from *E. coli* DH5 α .
1. 1.5 ml of *E. coli* DH5 α overnight culture was pelleted for 2 minutes at 10,000 rpm.
 2. The pellet was resuspended in 550 μ l TE buffer.
 - 10 3. 30 μ l 10 % SDS and 30 μ l of 20 mg/ml pronase were added.
 4. The tubes were mixed gently by inversion and incubated at 37°C for 1 hour.
 5. 100 μ l 5M NaCl solution was added and the solution was mixed for a few seconds.
 - 15 6. An equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added and the solution was mixed gently.
 7. The tubes were centrifuged at 11,000 rpm for 5 minutes.
 8. The upper phases were collected and an equal volume of Phenol:Chloroform (1:1) was added to each tube and mixed gently.
 - 20 9. The tubes were centrifuged at 11,000 rpm for 5 minutes and the upper phases were collected in fresh Eppendorf tubes.
 10. 200 μ l of silicon carbide 1000 slurry was added to each tube and mixed well.
 - 25 11. The tubes were centrifuged at 14,000 rpm for 1 minute.
 12. The supernatant was aspirated from each resin pellet.
 13. 500 μ l Wash Solution was added to each tube which was then vortexed well to mix.
 - 30 14. The tubes were centrifuged for 1 minute at 14,000 rpm.
 15. The wash was aspirated and repeated once more.
 16. The resin pellets were dried at 37°C for 5 minutes.
 17. TE buffer warmed to 65°C was added to a volume of 200 μ l to elute the genomic DNA.

- 15 -

In the above manner, four samples of *E. coli* DH5 α genomic DNA were isolated and run on agarose gel (Figure 4, lanes 1-4). Furthermore, two samples of *E. coli* DH5 α genomic DNA were isolated using isopropanol precipitation rather than the silicon carbide method of the invention. These two samples are shown in lanes 5 and 6 of Figure 4. These results indicate that genomic DNA may be isolated using silicon carbide and that such DNA isolated using the method of this invention is likely to contain less protein contamination than DNA using conventional methods of genomic isolation (note greater amount of proteins in wells 5 and 6 relative to wells 1-4).

Example 8

Isolation of RNA Using Silicon Carbide 1000

This example illustrates the capacity of silicon carbide 1000 to bind ribonucleic acids (RNAs). To demonstrate the ability of silicon carbide 1000 to bind RNA, the protocol outlined in Example 2 was carried out without the inclusion of 100 μ g/ml RNase A in the Cell Resuspension Solution. In this manner, RNAs liberated from *E. coli* DH5 α were not degraded and were isolated in the same fashion as plasmid DNA. This RNA is clearly visible in Figure 5.

Example 9

Isolation of a Range of Plasmid Sizes

This example illustrates that silicon carbide 1000 can be used to isolate plasmids of a range of sizes. Three plasmids (Table B) were isolated from overnight cultures of *E. coli*.

TABLE B. Plasmid Sizes

PLASMID	SIZE (bp)
pUC19	2686
pEGFP-N1	4733
PCMV beta	7164
pC BAV-KAN	36454

Following these isolations, plasmids were digested with restriction enzymes and run on agarose gel (Figure 6).

Example 10

5 Isolation of DNA Using Silicon Carbide Yields DNA of Quality Comparable to Commercial DNA Purification Kits.

This example briefly illustrates that plasmid DNA purified using the protocol described in Example 2 is comparable in quality to plasmids isolated using three commercial plasmid
10 purification kits. The plasmid used for these trials was pCMV beta (7164 bp). Following the isolation procedures, each preparation of plasmid was digested with the restriction enzyme *Hind* III and run with undigested samples on an agarose gel (Figure 7). Plasmid DNA isolated using silicon carbide
15 1000 demonstrated complete digestion with *Hind* III and appeared comparable to plasmid isolated using the three commercial kits used for comparison.

Example 11

20 Transformation of *E. coli* Cells with Silicon Carbide-Purified Plasmid DNA

This example illustrates the transformation of *E. coli* cells with DNA purified using the process presented in Example 2. Two plasmids were used for the transformation procedure: pUC19 and pCMV beta. Details of the results are presented in Table
25 C.

TABLE C: Transformation of *E. coli* using SiC-purified plasmid DNA

Plasmid	DNA (ng)	Colonies observed
pUC19	100	yes
pCMV beta	162	yes

Plasmid DNA purified using silicon carbide 1000 was found to be able to confer ampicillin resistance upon *E. coli* cells which previously lacked this genotype.

5 While only specific embodiments of the invention have been described, it is apparent that various additions and modifications can be made thereto, and various alternatives can be selected. It is, therefore, the intention in the appended claims to cover all such additions, modifications and
10 alternatives as may fall within the true scope of the invention.

Although in the preferred purification method of the invention as described above, a SiC grade of 1000 was used, other grain sites can be used. A range of 600 to 1000 is preferred. It
15 will be readily understood that SiC particles of smaller size provide an overall larger nucleic acid binding surface. However, extremely small particle sizes may result in excessive compacting of the SiC and insufficient mixing with the sample or the washing and elution media.

20 This invention is useful for isolating nucleotide polymers such as DNA, in a wide range of sizes, and whether the nucleotide polymer is single stranded or double stranded. The invention can be used for the isolation of either RNA or dNA. This simple method involves the principle steps of:

- 25 1. immobilizing the nucleotide polymer in the sample into silicon carbide;
2. separating the silicon carbide with the nucleotide polymer immobilized therein from the sample;
3. washing the silicon carbide-bound nucleotide polymer with
30 an ethanol-containing buffer;
4. removing the ethanol-containing buffer; and
5. eluting the nucleotide polymer in a lower salt buffer or water.

- 18 -

WE CLAIM:

1. The use of silicon carbide for binding a nucleotide polymer.
2. The use of silicon carbide according to claim 1 in which the nucleotide polymer is selected from the group consisting of a DNA oligonucleotide, a DNA polynucleotide, an RNA oligonucleotide, and an RNA polynucleotide.
3. The use of silicon carbide according to one of claims 1 and 2 in which the nucleotide polymer is a single-stranded chain.
4. The use silicon carbide according to one of claims 1 and 2 in which the nucleotide polymer is a double-stranded chain.
5. The use of silicon carbide according to one of claims 1-4 in which the nucleotide polymer is linear.
6. The use of silicon carbide according to one of claims 1-4 in which the nucleotide polymer is circular.
7. The use of silicon carbide according to one of claims 1-6 in which the silicon carbide has a mean grit size of 4.5 microns.
8. The use of silicon carbide according to one of claims 1-6 in which the silicon carbide has a mean grit size of 3 microns.
9. The use of silicon carbide according to one of claims 1-6 in which the silicon carbide has a mean grit size of 6.5 microns.
10. A method for the isolation of a nucleotide polymer from a sample comprising the steps of:
 - adding silicon carbide to the sample and mixing for binding of the nucleotide polymer to the silicon carbide;

separating the silicon carbide with the bound nucleotide polymer from the mixture obtained;

eluting the nucleotide polymer from the silicon carbide.

11. The method according to claim 10 using a spin column filter to separate the silicon carbide from the mixture.

12. The method of claim 10 in which the pore size of the spin column filter is in the range of .22 microns to 0.45 microns.

13. The method according to claim 10 using centrifugation to separate the silicon carbide from the mixture.

14. A method for the isolation of a nucleotide polymer from an agarose gel comprising the steps of:

excising a nucleotide polymer containing band from the agarose gel in solution;

placing the excised band in solution;

heating the solution for melting the excised agarose band;

adding silicon carbide to the solution for binding the nucleotide polymer thereto and mixing;

separating the silicon carbide and bound nucleotide polymer from the mixture;

eluting the nucleotide polymer from the silicon carbide.

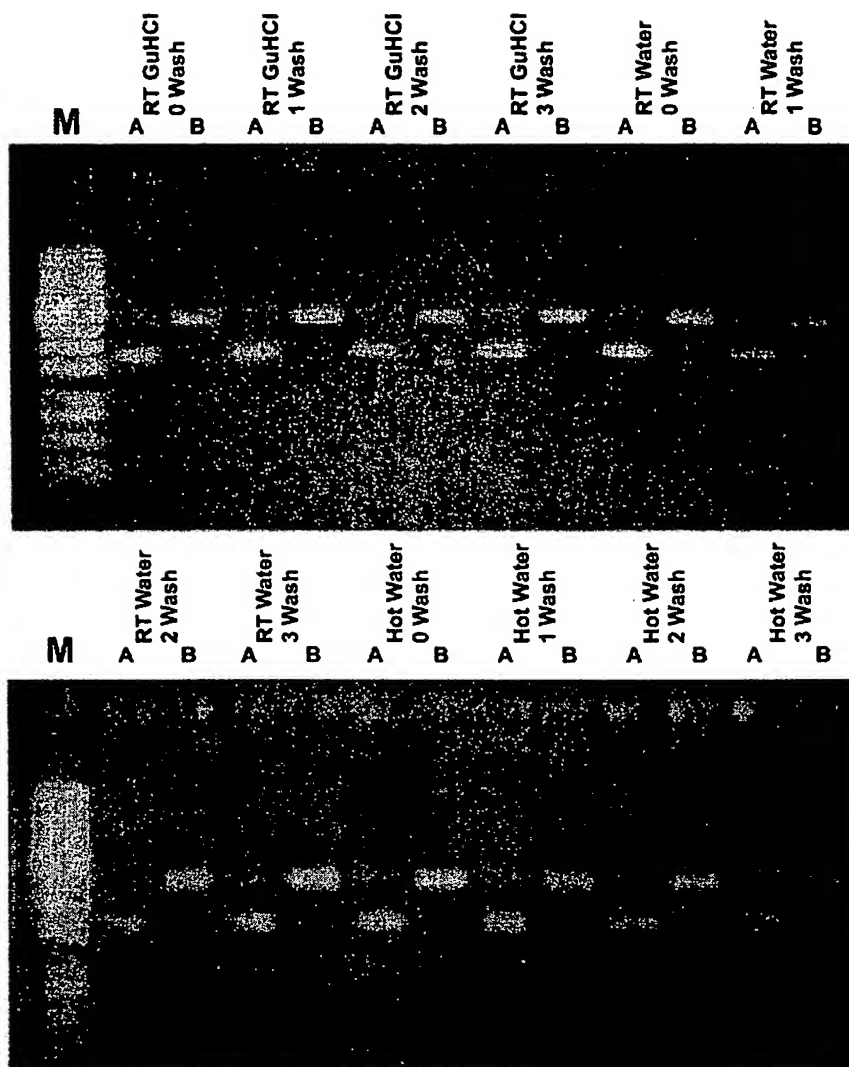
15. The method according to claim 14, wherein the solution containing the excised band is heated to 65°C.

16. The method according to claims 13 and 14 in which the DNA containing band excised from the agarose gel is placed in a guanidine hydrochloride solution.

17. The method according to claims 10-16 in which the nucleotide polymer is eluted with one of water, a low salt buffer, and TE buffer.

18. The method of claims 10-17 in which several washes of the silicon carbide are performed prior to elution of the bound nucleotide polymers.
19. The method of claim 10 in which the silicon carbide is added to the sample as a slurry.
20. The method of claim 19 in which the silicon carbide slurry is a 15% w/v mixture of silicon carbide in water or guanidine hydrochloride.

1/7

**FIG. 1**

Effect of pre-washing Silicon Carbide 1000 (SiC 1000) on quality of plasmid DNA isolated. SiC 1000 was treated before use with a variety of types and frequencies of washes. pUC 19 plasmid DNA was then isolated with the washed resin and digested with the restriction enzyme *Hind* III. Type and frequency of wash are indicated above each pair of lanes. "A" lanes show undigested pUC 19. "B" lanes show pUC 19 digested with *Hind* III. "M" lane is the marker lane. RT = room temperature. GuHCl = 1 M Guanidine Hydrochloride.

2/7

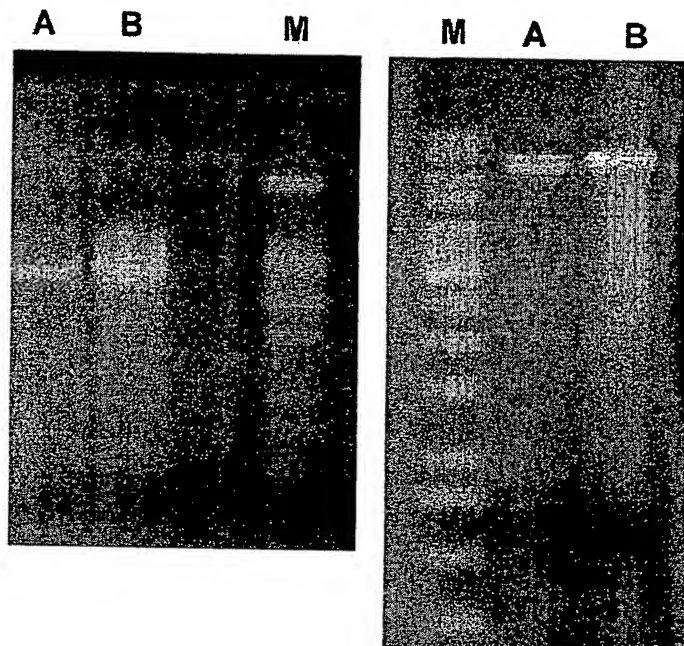
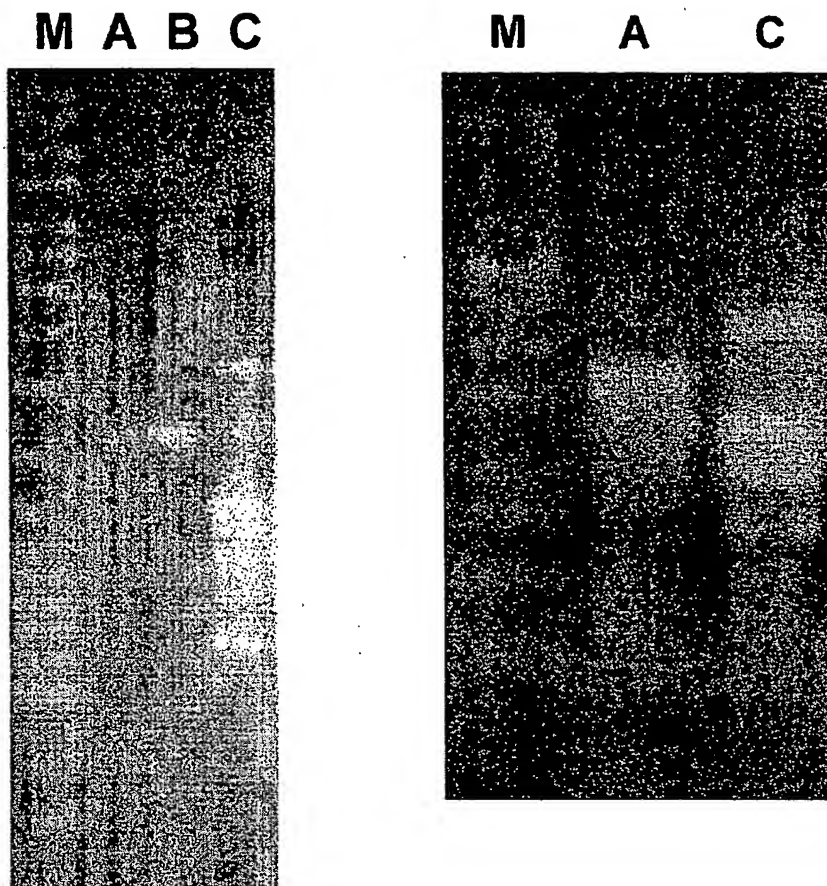


FIG. 2

Isolation of DNA fragments from agarose gel slices. pCMV beta (7164 bp) DNA was digested with the restriction enzyme *Hind* III which yielded the single linear fragment of pCMV beta DNA 7164 bp in length (left side photo). The DNA present in lanes A and B was isolated from agarose slices cut from the gel as described in Example 5. The isolated DNA was subsequently run on a second agarose gel and photographed to ascertain the recovery (right side photo). "M" lanes are marker lanes.

3/7

**FIG. 3**

PCR Product Isolation. PCRs were carried out and a sample of the reaction products were loaded onto agarose gel for analysis (left photo). PCR samples A and C were then used to test the ability of silicon carbide to isolate PCR fragments as described in Example 6. The isolated PCR fragments were then run on a second agarose gel for comparison (right photo). "M" represents a marker lane.

4/7

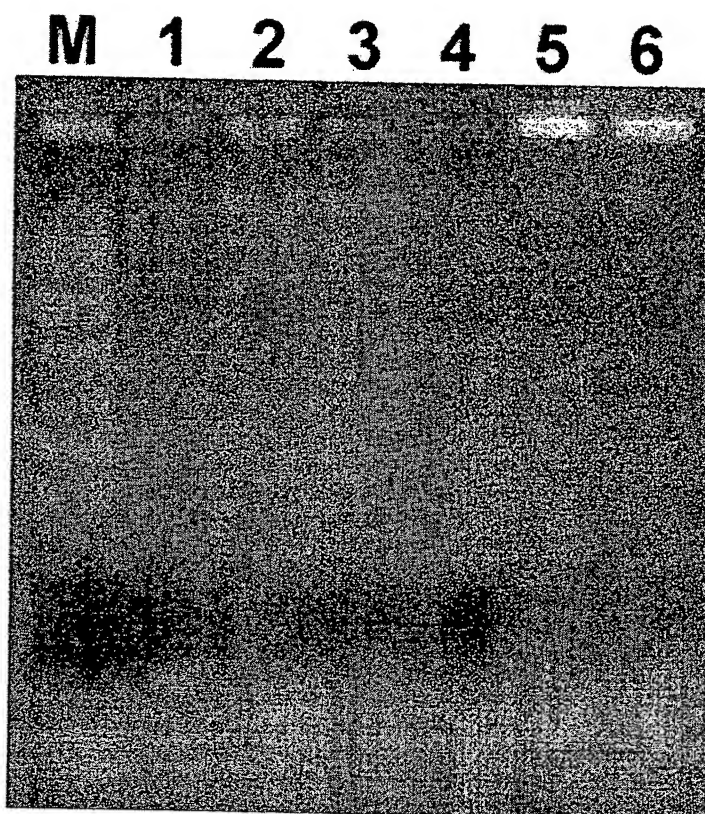


FIG. 4

Isolation of Genomic DNA from *E. coli* DH5 α . Lanes 1-4 show four samples of genomic DNA isolated as described in Example 7. Lanes 5 and 6 show genomic DNA isolated by isopropanol precipitation for comparison. Lane "M" is a marker lane.

5/7

Example 8: Isolation of RNA Using Silicon Carbide 1000

This example illustrates the capacity of silicon carbide 1000 to bind ribonucleic acids (RNAs).

To demonstrate the ability of silicon carbide 1000 to bind RNA, the protocol outlined in Example 2 was carried out without the inclusion of 100 µg/ml RNase A in the Cell Resuspension Solution. In this manner, RNAs liberated from *E. coli* DH5α were not degraded and were isolated in the same fashion as plasmid DNA. This RNA is clearly visible in figure 5.0.

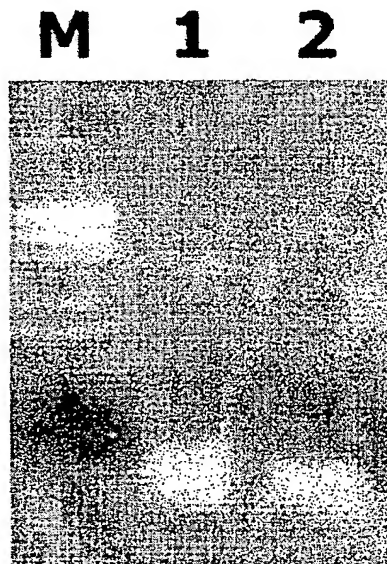
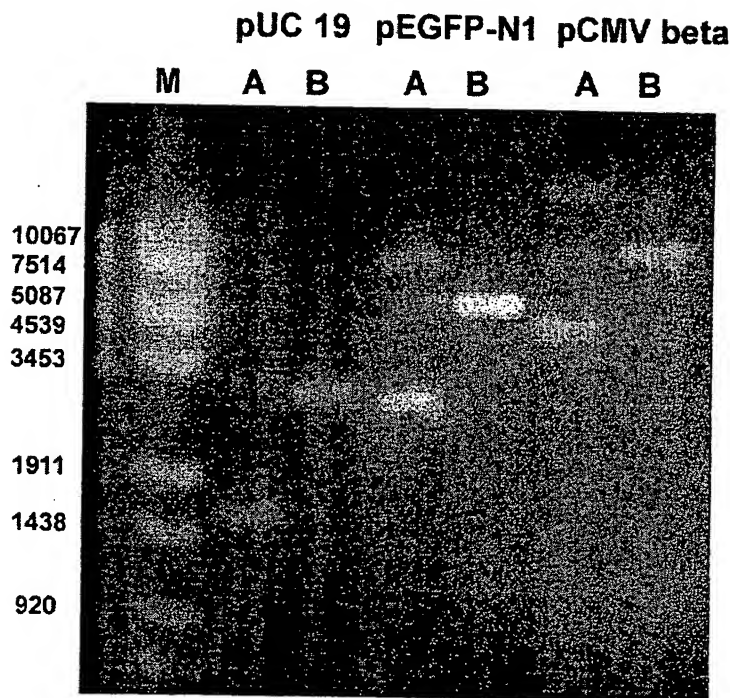


FIG. 5

Binding of RNA to silicon carbide 1000. Two samples of RNA were isolated using silicon carbide 1000 and run an agarose gel. Lanes 1 and 2 show RNA samples. Lane "M" is a marker lane.

6/7

**FIG. 6**

Isolation of a range of plasmid sizes using silicon carbide and digestion with restriction enzymes. "A" lanes show undigested samples. "B" lanes show digested samples. Plasmids are indicated at top of figure. "M" lane is the marker lane. pUC 19 was digested with *Hind* III. pEGFP-N1 was digested with *Bam* HI. pCMV beta was digested with *Hind* III.

7/7

**Example 10: Isolation of DNA Using Silicon Carbide Yields DNA of Quality
Comparable to Commercial DNA Purification Kits.**

This example briefly illustrates that plasmid DNA purified using the protocol described in Example 2 is comparable in quality to plasmids isolated using three commercial plasmid purification kits. The plasmid used for these trials was pCMV beta (7164 bp). Following the isolation procedures, each preparation of plasmid was digested with the restriction enzyme *Hind* III and run with undigested samples on an agarose gel (Figure 7.0). Plasmid DNA isolated using silicon carbide 1000 demonstrated complete digestion with *Hind* III and appeared comparable to plasmid isolated using the three commercial kits used for comparison.

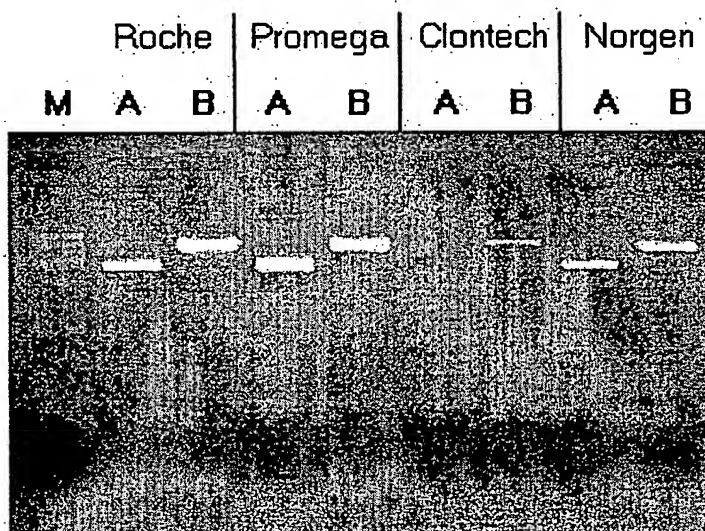


FIG. 7

Comparison of digestions of plasmid pCMV beta (7164 bp) purified using three commercial DNA purification kits and silicon carbide 1000. "A" lanes show undigested pCMV beta while "B" lanes show pCMV beta digested with *Hind* III. Lane "M" is a marker lane. Commercial kits used for purification are listed along top of figure.

INTERNATIONAL SEARCH REPORT

Int. national Application No

PCT/CA 00/00424

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 28148 A (ZENECA LTD) 8 December 1994 (1994-12-08) the whole document	1-6
A	EP 0 389 063 A (AKZO NV) 26 September 1990 (1990-09-26) the whole document	
A	WO 92 07863 A (DIAGEN INST MOLEKULARBIO) 14 May 1992 (1992-05-14) the whole document	
A	EP 0 648 776 A (BECTON DICKINSON CO) 19 April 1995 (1995-04-19) the whole document	
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

25 August 2000

Date of mailing of the international search report

01/09/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3018

Authorized officer

Oderwald, H

INTERNATIONAL SEARCH REPORT

Int ernational Application No

PCT/CA 00/00424

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>US 5 155 018 A (CUDDY KEVIN K ET AL) 13 October 1992 (1992-10-13) the whole document</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. Patent Application No.

PCT/CA 00/00424

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9428148	A	08-12-1994	AU 4339593 A	20-12-1994
EP 0389063	A	26-09-1990	NL 8900725 A	16-10-1990
			AT 156830 T	15-08-1997
			AU 641641 B	30-09-1993
			AU 5215390 A	27-09-1990
			CA 2012777 A	23-09-1990
			DE 69031237 D	18-09-1997
			DE 69031237 T	02-01-1998
			DE 389063 T	10-10-1996
			DK 389063 T	30-03-1998
			EP 0819696 A	21-01-1998
			ES 2085245 T	01-06-1996
			GR 96300019 T	31-03-1996
			GR 3025351 T	27-02-1998
			JP 2289596 A	29-11-1990
			JP 2680462 B	19-11-1997
			JP 10072485 A	17-03-1998
			KR 148693 B	01-08-1998
			US 5234809 A	10-08-1993
			ZA 9002190 A	24-12-1991
WO 9207863	A	14-05-1992	DE 4034036 A	30-04-1992
			DE 59105403 D	08-06-1995
			EP 0555270 A	18-08-1993
			US 5652141 A	29-07-1997
			US 6020186 A	01-02-2000
EP 0648776	A	19-04-1995	US 5503816 A	02-04-1996
			DE 69421885 D	05-01-2000
			DE 69421885 T	27-04-2000
			JP 7177886 A	18-07-1995
			US 5674997 A	07-10-1997
US 5155018	A	13-10-1992	NONE	

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☒ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.

THIS PAGE BLANK (USPTO)